(12)UK Patent Application (19)GB (11) 2012169 A

(21) Application No 7848859
(22) Date of filing
18 Dec 1978
(23) Claims filed
18 Dec 1978
(30) Priority data
(31) 53116/77
(32) 21 Dec 1977
(33) United Kingdom (GB)
(43) Application published
25 Jul 1979
(51) INT CL² A61L 13/00
A61K 31/19
(52) Domestic classification
A5E 247 274 279 500 501 506 509 510 A
A5B 170 180 190 285 28Y 38Y 390 401 40Y H
(56) Documents cited
GB 1505388
(58) Field of search
A5B
A5E
(71) Applicant
BP Chemicals limited
Britannic House
Moor Lane
London EC2Y 9BU
(72) Inventor
David Berry
(74) Agents
J Harry

(54) **Anti-viral compositions**

(57) The present invention relates to a method of disinfecting farm and domestic animals, and agricultural, domestic, hospital and industrial buildings (including their utilities) and equipment and land mass which may be exposed to or be the source of viral infections using a composition comprising a cation selected from NH^+_4 ion and ions of a metal from Group I or Group II of the Periodic Table due to Mendeleef, and formic acid, wherein the ratio of formic acid to cation is at least 2:1 on a chemical equivalent basis. The method of disinfection is particularly effective against viruses responsible for foot and mouth disease and swine vesicular disease. When applied to animals the compositions can be administered externally or internally, e.g. orally or in the form of a vaccine.

GB 2012169 A

SPECIFICATION

Compositions containing acid formates for use as anti-viral disinfectants

The present invention relates to compositions containing acid formates for use as anti-viral disinfectants.

Viruses are members of a group of sub-microscopic agents that infect animals and plants, usually manifesting their presence by causing disease. They are distinct from bacterial organisms which are microscopic as distinct from sub-microscopic. Of particular concern are the viral diseases such as foot and mouth disease and swine vesicular disease in animals and those which attack plants, especially the Angiosperms. Such viral diseases are spread in animals by insect vectors by contact or as droplets of mucus expelled from the nose, mouth and throat of the infected animal which are inhaled by another animal. Viral diseases in plants are transmitted by insects such as aphids and leaf-hoppers or by other means of transfer. Outbreaks of foot and mouth disease and swine vesicular disease are countered in many countries by the slaughter of infected animals, mainly as a means of eradicating the disease and as a preventative measure because of the shortcomings of the disinfectants available hitherto. Those that are available can be obnoxious to the administering operatives, corrosive to the equipment at the concentrations recommended or unsuitable if there is a risk of their contaminating animal feeds or drinking water and are therefore unsatisfactory in use.

It has now been found that these problems may be minimised by using compositions containing acid formates of the type claimed and described in our British patent specification Serial No. 1 505 388.

Accordingly the present invention is a method of disinfecting animals and/or articles infected by or susceptible to viral infections comprising applying to said animal or article a composition comprising a cation selected from NH^+_4 ion and ions of a metal from Group I or Group II of the Periodic Table due to Mendeleef, and formic acid, wherein the ratio of formic acid to cation is at least 2:1 on a chemical equivalent basis.

The animals infected by or susceptible to infection mainly include farm and domestic animals such as the equine species, cattle, sheep, pigs and poultry. The term "articles" as used herein and throughout the specification includes agricultural, domestic, hospital and industrial buildings (including their utilities) and equipment and land mass which may be exposed to or be the source of viral infections.

The Group I and Group II metals of the Periodic Table due to Mendeleef are preferably selected from sodium, potassium, calcium and magnesium. The chemical equivalent ratio of acid to cation is preferably between 4:1 and 8:1.

The compositions for use as disinfectants preferably contain the cations and formic acid in the form of associated species which for the sake of convenience will hereafter be termed as "acid formate", such as for instance a tetraformate.

The compositions may contain one or more of these acid formates. For example, the compositions may contain ammonium diformate, potassium diformate, ammonium tetraformate, sodium tetraformate, magnesium tetraformate and calcium tetraformate. In the case of the calcium and magnesium tetraformates, by virtue of the cation being divalent, it represents one equivalent of Ca^{++} or Mg^{++} reacting with two equivalents or formates.

The acid formates may be prepared by mixing formic acid with a calculated amount of a base of the desired cation in an aqueous medium or alternatively by mixing the neutral formate with a calculated amount of the free acid. For example, in preparing compositions containing the ammonium ion the acid may be mixed with a concentrated aqueous ammonia solution. On the other hand, for preparing compositions containing the calcium ion, a neutral calcium formate of the acid may be dissolved in an appropriate amount of the free acid or the free acid may be partially neutralised by lime or reacted with limestone.

In disinfecting the animals and articles, the acid formate is preferably used as an aqueous solution. The concentration of the acid formate in aqueous solution will depend upon the particular viral infection to be controlled and the intensity of the infection. For example, when disinfecting domestic, hospital, industrial or agricultural areas, a concentration of between 0.1 and 5% w/w in aqueous solution may be sufficiently effective. For treating infected animals and tissues it is preferable to use a slightly more concentrated solution e.g. up to 10% w/w of the aqueous solution. In treating particularly virulent infections a concentrated solution of the formate containing up to 70% of the acid formate may be used. In certain cases treatment may have to be repeated more than once for best effect.

In the method of the present invention the compositions used to disinfect may contain other conventional additives. In particular, the compositions may contain other additives such as acetic acid, propionic acid, citric acid, lactic acid, glycollic acid, iodophores, formalin, benzoic acid, salicylic acid, ethanol, chloroxylenol, detergents, wetting agents, and anti-foam agents.

The method of disinfection is particularly effective against viruses responsible for foot and

mouth disease (FMD) and swine vesicular dis ase (SVD). Oth r virus s which may be inactivated to som ext nt by th c mpositions of this invention includ those responsibl for Newcastle Disease, Rabies, Swine fev r, African Swine fever, Tesch n Disease, Talfon Dis as , Aujesky's Diseas , Equine Rhinopneumonitis, Enteroviruses, Blu Tongue Disease, infectious Bovine Rhinotracheitis, and those plant viruses responsible for e.g. tobacco mosaic disease which affects tobacco, potato and tomato plants. It is known that the major pig viruses e.g. SVD have high acid stability and can be unaffected by pH levels as low as pH 2.0. Surprisingly it has been found that the compositions employed in the method of the present invention inactivate SVD virus at pH levels as high as pH 3.1, as is illustrated in the Examples. The compositions may be applied to the animals and articles by any of the conventional methods e.g. by spraying, dipping, brushing or daubing. These compositions are relatively safe to handle and have relatively low corrosivity and are effective against both bacterial and viral infections. Accidental ingestion of these compositions in moderate amounts do not harm farm animals; indeed, they have been used as feed additives for such animals. The compositions may also be therapeutically used in such animals particularly if the viral infection is localised in the digestive tract of the animal. The dosage in the latter context will naturally be a therapeutic dosage depending upon the nature of the infection and the size of the animal.

In therapeutic use the compositions may be introduced into the animal either orally or in the form of vaccines during the preparation of which they may be used to inactivate the virus culture.

The present invention is further illustrated with reference to the following Example.

Examples

Example 1

Inactivation of Foot and Mouth Disease Virus

Using a temperature of 10°C throughout 0.2 cm^3 of a suspension of foot and mouth disease virus was added to 20 cm^3 of the following solutions:

Phosphate Buffered Saline (PBS pH 7.6)
Phosphate Buffered Saline + 0.85% w/w formic acid
Phosphate Buffered Saline + 1.70% formic acid
Phosphate Buffered Saline + 3.40% formic acid
Phosphate Buffered Saline + 3.0% ammonium tetraformate

The formic acid and ammonium tetraformate were added as the 85% and 75% aqueous solutions respectively. After 30 minutes at 10°C, the solutions were brought approximately to pH 7.6 by addition of 1N sodium hydroxide solution, and 1.0% Ox Serum was added. Virus assays were carried out on RS2 tissue culture cells. Each experiment was done in duplicate.

Results of these experiments are presented below, where the virus assays are expresses as total virus in assay preparation mixture.

Medium of Incubation	pH before Neutralisation	Total Virus In Assay Preparation	Survival
PBS	7.61	4.4×10^6	100.0
PBS + 0.85% Formic Acid	2.21	$<3.05 \times 10^1$	<0.0007
PBS + 1.70% Formic Acid	2.05	$<3.58 \times 10^1$	<0.00081
PBS + 3.40% Formic Acid	1.80	$<4.7 \times 10^1$	<0.0011
PBS + 3.0% Ammonium Tetraformate	3.01	$<3.7 \times 10^2$	<0.0084

All the above treatments would be considered as being completely effective in destroying FMD virus, and it would be anticipated from measurements of pH that 1.50% ammonium t traformate (2.0% w/w of 75% w/w aqueous solution) would also give complete virus control.

Example 2

Inactivation of Swine Vesicular Disease Virus

At 10°C throughout, 1 cm^3 of a suspension SVD virus was diluted into 100 cm^3 of the following solutions:

Phosphat Buff red Saline

Phosphate Buff red Saline + 0.85% w/w f rmic acid
Phosphate Buffered Saline + 1.70% w/w formic acid
Ph sphate Buffered Saline + 3.40% w/w formic acid
Phosphate Buffered Saline + 1.50% w/w ammonium tetraformate
Phosphate Buffered Saline + 3.0% w/w ammonium tetraformate

After 2 or 4 days at + 10°C, virus from each preparation was diluted in 1/10 steps into PBS (pH 7.25). Assays were carried out on plates of RS tissue culture cells which first had to be re-neutralised with a minimal volume of sodium hydroxide.

Results of the above experiments are presented below.

Medium of Incubation	pH 2 days	pH 4 days	Plaque Forming Units/ml of Reaction Mixture 2 days	Plaque Forming Units/ml of Reaction Mixture 4 days	% Survival 2 days	% Survival 4 days
PBS	—	7.30	—	9.4×10^7	—	104.4
PBS + 0.85% Formic Acid	—	2.36	—	2.25×10^3	—	0.0025
PBS + 1.70% Formic Acid	2.12	2.12	$<1.79 \times 10^*$	$<1.25 \times 10^2$	<0.000002	<0.00014
PBS + 3.4% Formic Acid	1.90	1.88	$<2.34 \times 10^*$	$<1.25 \times 10^2$	<0.0000026	<0.00014
PBS + 1.50% Ammonium Tetraformate	3.11	3.09	2.91×10^5	3.92×10^4	0.32	0.044
PBS + 3.0% Ammonium Tetraformate	3.08	3.07	1.03×10^3	$<1:25 \times 10^2$	0.0011	<0.00014

Thus reductions in virus numbers were achieved by formic acid in th range 0.85–3.40% by weight and by ammonium tetraformat in th range 1.50–3.0% by w ight. However, for practical applications, adequat control would be given by addition of 1.70% formic acid and 3.0% ammonium tetraf rmate.

Example 3

Experiments to test the effects of aqueous formic acid (85%) and aqueous ammonium tetraformate (ATF, 75%) on the viruses of foot and mouth disease and swine vesicular disease in the tissues of affected animals were carried out by adding these to suspensions of infected tissue in phosphate buffered saline (PBS) to give concentrations of 1.70% formic acid and 3% ATF in the final suspensions.

The tissues used were skin, muscle, liver and lymph node.

In Experiment A all tissues were used from freshly slaughtered animals (3 pigs). In Experiments B–E tissues had been stored at − 20°C for varying periods. The results of each experiment are tabulated below in which a dashed line (–) indicates that none was detected and the abbreviation "ND" indicates that the test was not done.

Experiment A

Inactivation of swine vesicular disease virus in skin, muscle and lymph node of pigs 1–3 and in the kidney of pig 2 was complete within 3–24 hours.

There was a persistence of infection in liver samples of all 3 pigs in the presence of aqueous formic acid and ammonium tetraformate.

Experiment B

A repeat test with the frozen samples of liver indicated unsatisfactory inactivation of virus.

Experiment C

Swine vesicular disease virus (SVDV) in suspension was not inactivated by aqueous formic acid or ammonium tetraformate in the presence of minced normal liver tissue. In contrast control experiments involving suspensions of SVD Virus in phosphate buffered saline did give inactivation with formic acid and ATF.

Experiments D–E

Foot and mouth disease virus (FMDV) in skin and lymph node was inactivated by both aqueous formic acid and ammonium tetraformate. The presence of minced liver did not affect the action of aqueous formic acid or ATF on FMD Virus.

EFFECT OF FORMIC ACID AND ATF ON SVD VIRUS IN PIGS TISSUE

Log_{10} (Plaque Forming Units/ml)

Expt. No A

		Skin			Muscle			Liver			Kidney			Lymph Node		
		PBS	Formic Acid	ATF	PBS	Formic Acid	ATF	PBS	Formic Acid	ATF	PBS	Formic Acid	ATF	PBS	Formic Acid	ATF
	0	–			2.4			–			–			1.8		
Pig	3	2.8	–	1.7	–	2.6	2.8	–	3.3	2.5	–	–	1.7	2.9	1.7	–
	Hours 24	3.0	–	–	2.6	–	2.0	–	3.4	2.7	–	–	–	2.7	–	–
1	48	2.7	–	–	3.2	–	–	–	2.7	–	–	–	–	2.0	–	–
	72	2.5	–	–	–	–	–	–	2.8	–	–	–	–	2.5	–	–
	0	2.0			1.4			2.3			3.0			1.4		
Pig	3	2.7		2.7	–	2.8	2.7	3.8	3.5	2.7	2.9	2.5	1.7	3.1	2.7	1.0
	Hours 24	2.5	–	–	2.8	–	–	3.5	–	–	3.2	–	–	–	–	–
2	48	2.8	–	–	2.7	–	–	3.9	–	2.0	2.7	–	–	3.2	–	–
	72	2.7	–	–	2.4	–	–	3.6	–	2.5	3.1	–	–	3.3	–	–
	0	–			1.7			2.4			–			1.4		
Pig	3	–	–	–	2.4	–	–	2.6	–	–	–	–	–	2.8	–	–
	Hours 24	–	–	–	1.7	–	–	3.5	–	2.7	–	–	–	2.9	–	–
3	48	–	–	–	1.7	–	–	2.6	–	2.2	–	–	–	2.6	–	–
	72	–	–	–	2.0	–	–	2.7	–	–	–	–	–	–	–	–

Expt. No B
EFFECT OF FORMIC ACID AND ATF ON THE VIRUS OF SWINE VESICULAR DISEASE IN PIGS LIVER.
Log $_{10}$ (PLAQUE FORMING UNITS/ml)

	Hours	PBS	Formic Acid	ATF
	0	3.0		
	3	2.4	2.5	2.7
Pig 1	24	1.7	2.3	2.0
	48	2.0	1.7	2.3
	72	1.7	1.7	1.7
	0	2.0		
	3	2.5	1.7	1.7
Pig 2	24	ND	ND	ND
	48	ND	ND	ND
	72	2.0	1.7	2.0
	0	3.5		
	3	2.9	ND	2.9
Pig 3	24	ND	ND	ND
	48	ND	ND	ND
	72	2.8	ND	2.0

Expt. No C
EFFECT OF MINCED LIVER ADDED TO A SUSPENSION OF SVDV IN PBS CONTAINING EITHER FORMIC ACID OR ATF Log $_{10}$ (PLAQUE FORMING UNITS/ml)

Hours	PBS + Liver	Control PBS
0	4.8	4.9
3	5.1	5.2
24	5.4	4.7
48	5.1	5.2
72	5.1	4.8

Hours	Liver PBS/ Formic Acid	Control PBS/ Formic Acid
0	–	
3	4.3	<1.7
24	4.7	<1.7
48	4.5	<1.7
72	4.4	<1.7

Hours	Liver PBS/ ATF	Control PBS/ ATF
0	–	
3	4.8	3.2
24	4.8	1.7
48	5.0	<1.7
72	4.9	<1.7

Expt. No D
EFFECT OF MINCED LIVER ON THE ACTION OF FORMIC ACID AND AMMONIUM TETRAFORMATE ON FMDV
Log $_{10}$ (PLAQUE FORMING UNITS/ml)

Hours	PBS/PBS	PBS/Liver
0	3.5	4.1
24	3.6	3.4
48	4.2	2.9
72	3.1	3.1

	Formic Acid/ PBS	Formic Acid/ Liver
0	–	–
24	<1.7	<1.7
48	<1.7	<1.7
72	<1.7	<1.7

Hours	ATF/ PBS	ATF/ Liver
0	–	–
24	<1.7	<1.7
48	<1.7	<1.7
72	<1.7	<1.7

Expt. No E
EFFECT OF BIOCIDE AND AMMONIUM TETRAFORMATE ON FMDV IN AFFECTED PIG TISSUES
Log$_{10}$ (PLAQUE FORMING UNITS/ml)

	Hours	PBS	Formic Acid	ATF
SKIN	0	3.7		
	24	3.2	–	–
	48	2.7	–	–
	72	1.8	–	–
LIVER	0	–		
	24	–	–	–
	48	–	–	–
	72	–	–	–
LYMPH NODE	0	1.7		
	24	2.2	–	–
	48	2.2	–	–
	72	1.7	–	–

CLAIMS

1. A method of disinfecting animals and/or articles infected by or susceptible to viral infections comprising applying to said animal or article a composition comprising a cation selected from ammonium ion and ions of a metal from Group I or Group II of the Periodic Table du to Mendel f, and formic acid, wherein the ratio of formic acid to cation is at least 2:1 on a chemical quivalent basis.

2. A m th d according to claim 1 wher in th cation is s l cted fr m sodium, potassium, calcium and magn sium.

3. A method according to any of the preceding claims wher in th ratio of acid to cation is between 4:1 and 8:1.

4. A method according to any of the preceding claims wherein said composition contains on or more complex acidic formates selected from ammonium diformate, ammonium t traformate, sodium tetraformate, magn sium tetraformate and calcium tetraformate.

5. A method according to any of the preceding claims wherein said composition is applied as an aqueous solution.

6. A method according to claim 5 wherein the acid formate is applied at a concentration of between 0.1 and 5% w/w in aqueous solution.

7. A composition according to any of the preceding claims wherein the said composition contains in addition one or more of the additives selected from acetic acid, propionic acid, citric acid, lactic acid, glycollic acid, iodophores, formalin, benzoic acid, salicyclic acid ethanol, chloroxylenol, detergents, wetting agents and anti-foam agents.

8. A method of disinfecting animals and/or articles infected by or susceptible to viral infections according to claim 1 wherein the virus responsible for the infection is one or more of Foot and Mouth Disease, Swine Vesicular Disease, Newcastle Disease, Rabies, Swine Fever, African Swine Fever, Teschen Disease, Talfon Disease, Aujesky's Disease, Equine Rhinopneumonitis, Enterovirus, Blue Tongue Disease, infectious Bovine Rhinotracteitis, and Tobacco Mosaic Virus.

Printed for Her Majesty's Stationery Office by Burgess & Son (Abingdon) Ltd.—1979.
Published at The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.